Seed trait evaluation of *Gossypium barbadense* L. chromosomes/arms in a *G. hirsutum* L. background

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Abstract Investigation of cotton nutritional components is important because its seeds provide a useful nutritional profile and can possibly serve as a biofuel resource. In this study, five cultivars, 13 cotton chromosome substitution (CS-B) lines, their donor parent, '3-79', and their recurrent parent, 'TM-1', were evaluated for seed traits over four environments. A mixed linear model approach with the jackknife method was employed to estimate variance components and to predict genotypic effects for each seed trait. Genotypic effects were more important than genotype by environment interaction for all seed traits. Chromosome associations with these seed traits were detected using the comparative method by comparing the differences between each CS-B line and TM-1. For example, chromosome 4 of 3-79 in TM-1 background was associated with reduced seed index (SI), embryo percentage, protein percentage while associated with increased seed oil percentage and seed fiber percentage. Other chromosome associations with these seed traits were also observed in this study. SI was highly correlated with three seed index traits: seed protein index, seed oil index (OI), and seed fiber index. Lint percentage, boll number, and lint yield were positively correlated with protein percentage while negatively correlated with SI and OI. SI and seed fiber content exhibited negative correlations with micronaire but positive correlations with fiber length and strength. Results suggested that agronomic traits and seed nutrition components can be improved simultaneously.

Keywords Chromosome substitution line · Cotton · Seed traits · Chromosome associations

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Abbreviations

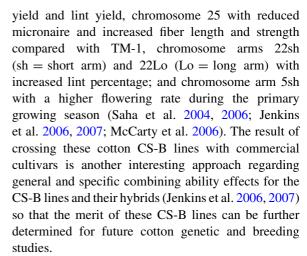|	
CS-B	Chromosome substitution line from *G. barbadense*
SI	Seed index
EP	Seed embryo percentage
PP	Seed protein percentage
OP	Seed oil percentage
FP	Seed fiber percentage
PI	Seed protein index
OI	Seed oil index
FI	Seed fiber index

Introduction

Cotton *Gossypium hirsutum* L., as a dual-use crop, provides natural fiber to the textile industry and nutrition components for both humans and livestock and played a key role in American agriculture for more than 200 years. Fiber quality has become increasingly important to the textile industry due to technology changes in cotton fiber spinning. Breeders endeavor to improve fiber quality while cottonseed usually is considered a by-product of lint production with limited breeding emphasis placed on important nutrition components such as seed oil and protein (Cherry et al. 1978a, b; Belyea et al. 1989). However, cotton seed has a good nutrition profile and a better understanding of the genetics and breeding of cottonseed traits and their relation to lint production should be worthy of investigation.

Commercial upland cotton cultivars produce 40–43% lint, thus around 57–60% seed. Among a wide range of cotton lines, oil content ranges from 17 to 27%, while protein ranges from 12 to 32%, ash 4 to 5%, and moisture 5 to 10% (Turner et al. 1976; Cherry et al. 1978a, b; Kohel et al. 1985; Belyea et al. 1989; Blasi and Drouillard 2002; Liu et al. 2002). Cottonseed crude oil which is refined to edible oil can be considered an important biofuel resource (Karaosmanoglu et al. 1999; Allen and Watts 2000; Meneghetti et al. 2007). In addition, cottonseed meal is used to feed sheep, dairy, and beef cattle, and other ruminant livestock (Blasi and Drouillard 2002). Cottonseed meal was reported to be better than soybean meal for feeding catfish (Barros et al. 2000). Limited genetic information has been reported for cottonseed nutrition components and physical properties (Bourland and Bird 1983; Dani and Kohel 1988; Wu et al. 1995; Wang et al. 1996a, b).

A set of cotton chromosome substitution (CS-B) germplasm lines have been released recently (Stelly et al. 2005). Each CS-B line has an individual chromosome or chromosome arm from 3-79 (*G. barbadense* L.) substituted into TM-1 (*G. hirsutum*). Each CS-B line is near-isogenic to the recurrent parent TM-1 with one chromosome or chromosome arm different. With such a high level of uniform genetic background, chromosome associations with traits of importance in cotton have been detected through the comparative analyzes. For example, chromosomes 16 and 18 from 3-79 in TM-1 background were associated with reductions in seed cotton

yield and lint yield, chromosome 25 with reduced micronaire and increased fiber length and strength compared with TM-1, chromosome arms 22sh (sh = short arm) and 22Lo (Lo = long arm) with increased lint percentage; and chromosome arm 5sh with a higher flowering rate during the primary growing season (Saha et al. 2004, 2006; Jenkins et al. 2006, 2007; McCarty et al. 2006). The result of crossing these cotton CS-B lines with commercial cultivars is another interesting approach regarding general and specific combining ability effects for the CS-B lines and their hybrids (Jenkins et al. 2006, 2007) so that the merit of these CS-B lines can be further determined for future cotton genetic and breeding studies.

In this study, seed nutritional components and seed physical traits of interest were measured in five cultivars, 13 cotton chromosome substitution lines, and their donor and recurrent parents (3-79 and TM-1) using four environments. The objectives were (1) to determine the difference between 13 CS-B lines and five cultivars; (2) to determine chromosome associations with seed traits using the comparative analyzes; and (3) to investigate the genetic correlations between agronomic and fiber traits and seed traits. The results obtained in this study should provide valuable information for both the genetic understanding of cotton seed traits and improvements of these traits in cultivar development.

Materials and methods

Materials and data collection

Thirteen near-isogenic euploid ($2n = 52$) BC_5S_2 chromosome substitution (CS-B) lines containing different pairs of *G. barbadense* chromosomes or segments, their recurrent parent, TM-1, donor parent, 3-79, and five commercial cultivars, 'Deltapine 90' (DP90; PVP 8100143), 'Sure-Grow 747' (SG747; PVP 9800118), 'Phytogen 355' (PSC355; PVP 200000167), 'Stoneville 474' (ST474; PVP 9400152), and 'FiberMax 966'(FM966; PVP200100209) were used in this study. In each CS-B line, a single chromosome pair of TM-1 has been replaced by the corresponding part of the 3-79 genome. Lines were designated by the chromosome number specific to the introgressed chromosome or chromosome arm of the alien species as follows:

CS-B02, CS-B04, CS-B06, CS-B07, CS-B16, CS-B17, CS-B18, CSB-25, CS-B05sh, CS-B14sh, CS-B15sh, CS-B22sh, and CS-B22Lo. The PI numbers of these lines have been reported previously (Stelly et al. 2005).

Development of these CS-B lines has been described in previous studies (Saha et al. 2004; Stelly et al. 2005). TM-1 is an inbred line derived from the commercial cultivar Deltapine 14 (PI 528970) and maintained over 40 generations by selfing (Kohel et al. 1970). The 75 top crosses were made at Mississippi State, MS, in the summer of 2002. The F_1 seeds were sent to a winter nursery in Tecoman, Mexico, to produce the F_2. The resulting 75 F_2 hybrids, five cultivars, 13 CS-B lines, TM-1 and 3-79 parents were planted at two sites in 2003 and 2004 at the Plant Science Research Center at Mississippi State, MS (33.4N 88.8W). Soil type for site one in 2003 was a Marietta loam (Fine-loamy, siliceous, active, fluvaquentic Eutrudepts) and for site two in 2003 and two sites in 2004 was a Leeper silty clay loam (Fine, smectitic, nonacid, thermic Vertic Epiaquept). Plots were planted in a plant two-skip one row pattern with single 9 m rows on site one in 2003 and 12 m rows on site two in 2003 and two sites in 2004, replicated four times in randomized complete block arrangements. Rows were 0.97 m apart and plants were spaced approximately 10 cm apart within the row. Planting dates were 28 May for 2003 and 13 May for 2004. Harvest dates were 3 November and 31 October for sites one and two in 2003, respectively, and 2–9 November and 29 October for sites one and two in 2004, respectively (Jenkins et al. 2006). A 25-boll sample was hand harvested from first position bolls near the middle nodes of plants in each plot before machine picking. Each sample was weighed and ginned on a laboratory 10-saw gin to determine boll weight (BW) and lint percentage (LP). Boll number per hectare was calculated by dividing seed cotton yield by boll weight (Tang et al. 1996). The lint samples were sent to STARLAB, Inc. in Knoxville, Tennessee, for determination of micronaire (MIC), elongation (E1), 2.5% span length (SL), and fiber strength (T1). Fiber analyzes were conducted by single instruments. However, only parental lines were used in this study.

Acid-delinted seed samples from four replications for each parental line in each environment (combination of year and site, defined in the original experiment) were mixed and used for further

analyzes. A 100 acid delinted-seed sampled from the mixed seed was used to measure seed index (SI) and then seed volume was determined by displacement of these seeds in Ethyl alcohol. Five to ten grams of seed for each line was cut by scissors and the embryo was removed by a needle to determine embryo weights, hull weights, embryo percentage (EP), and hull percentage. Twelve grams of acid delinted seeds for each genotype and environment were sent to Mississippi State Chemical Laboratory, Mississippi State, Mississippi, to determine seed percentage traits for protein (PP), oil (OP), fiber (FP), ash (AP), and moisture (MP). Determination of seed nutrition components followed the Official Methods of Analysis of AOAC International (Crude Protein: AOAC 990.03; Crude Fat: AOAC 920.39; Moisture: AOAC930.15; Ash: AOAC 942.05; and Crude fiber: AOAC 962.09) (Cunniff 1995). Seed index traits for protein (PI), oil (OI), fiber (FI), ash (AI), and moisture (MI) were determined by multiplying seed index with their content percentages.

Genetic model and data analysis

The phenotypic value of the ith genotype in the jth location and hth year is expressed as follows.

$$y_{hij} = \mu + E_h + G_i + GE_{hi} + L_{j(h)} + e_{hij}$$

where E_h is the year effect (h = 1, 2), G_i is the genotypic effect (i = 1, …, 20) GE_{hi} is the $G \times E$ interaction effect, $L_{j(h)}$ is the site effect within year, and e_{hij} is the random error. The above linear model could be considered a two-way factorial experiment design as site was treated as replication within year. Since we are interested in both genetic variations and genotypic effects, the mixed linear model approach, minimum quadratic unbiased estimation (MINQUE) approach was applied to estimate variance components and to predict effects of interest (Zhu 1989, 1993). The jackknife procedure was applied through consecutive removal of one observation (Miller 1974). An appropriate t-test was used to detect the significance for each parameter (one-tailed test for variance components and two-tailed for predicted effects). By this method, the predicted genetic effects were deviations from the respective population grand mean μ, not from TM-1. However, a significant difference for genetic effects between a specific CS-B

line and TM-1 can be calculated and considered as a significant chromosome effect due to the specific substituted chromosome or chromosome arm from 3-79 (Saha et al. 2004; Jenkins et al. 2006). The comparative method was used to detect significance chromosome associations through comparing each CS-B effects and TM-1 effects by the interval test (Saha et al. 2004; McCarty et al. 2006). Parent mean values for seed traits, agronomic traits, and fiber traits over four environments were used to calculate correlation coefficients with SAS CORR procedure (SAS Institute Inc 2003).

Results

Mean values of seed traits

Mean seed percentage traits over environments for protein, oil, ash, moisture, and fiber were 20.15, 19.87, 3.74, 8.04, 19.53%, respectively. Mean seed index traits for protein, oil, ash, moisture, and fiber were 1.98, 1.96, 0.37, 0.79, and 1.93 g, respectively. Mean seed volume, seed index, and embryo percentage were 9.90 ml, 9.85 g, and 63.50%, respectively. Since seed index and seed volume were highly correlated (0.95) and ash and moisture are not important seed nutrition component traits, we only report the results of seed index, embryo percentage, seed percentage and index traits for protein, oil, and fiber regarding their variance components, genotypic effects, and correlations between these traits.

Variance components

Variance components estimates expressed as proportions to the phenotypic variances for eight cotton seed nutritional and physical traits are summarized in Table 1. Genotypic effects were significant for all eight seed traits, while genotype by environment interaction effects were significant for SI, OP, EP, and OI (Table 1). Genotypic effects were mainly responsible for the phenotypic variances for seed index (86.2%). PI, OI, and FI showed more genotypic variations than PP, OP, and FP (61.8 vs. 17.7%, 70.0 vs. 45.1%, and 75.2 vs. 24.4%, respectively) (Table 1). This was probably due to large genetic contribution to the phenotypic variance for SI. Thus, it seems that in this study, nutrition components expressed as an index better describes genetic variations than nutrition components expressed as a percent. Contribution of residual effects to the phenotypic variance varied from trait to trait.

Predicted genotypic effects

Cotton researchers are not only interested in genetic variations in seed traits but genotypic effects as well. The genotypic effects plus each grand mean represents the genotypic values for all parents. The predicted genotypic effects for eight seed traits are presented in Table 2. On average, the five cultivars had higher PP than the 13 CS-B lines, TM-1, or line 3-79, while there were no obvious patterns between CS-B lines and cultivars for other seed traits. Line

Table 1 Variance component estimates expressed as proportions to phenotypic variances for cotton seed nutritional and physical traits

	SI ± SE	EP ± SE	PP ± SE	OP ± SE
V_G/V_P	0.862 ± 0.005	0.374 ± 0.016	0.178 ± 0.019	0.451 ± 0.013
V_{GE}/V_P	0.013 ± 0.005	0.209 ± 0.021	0.000 ± 0.000	0.162 ± 0.018
V_e/V_P	0.125 ± 0.005	0.417 ± 0.016	0.822 ± 0.042	0.387 ± 0.015
	FP ± SE	PI ± SE	OI ± SE	FI ± SE
V_G/V_P	0.244 ± 0.019	0.618 ± 0.011	0.700 ± 0.010	0.752 ± 0.010
V_{GE}/V_P	0.016 ± 0.030	0.004 ± 0.018	0.112 ± 0.012	0.008 ± 0.010
V_e/V_P	0.739 ± 0.026	0.378 ± 0.019	0.188 ± 0.007	0.239 ± 0.010

V_G, V_{GE}, V_e, and V_P are genotypic variance, genotype by environment interaction variance, random error variance, and phenotypic variance, respectively

SI seed index, *EP* embryo percentage, *PP* protein percentage, *OP* oil percentage, *FP* fiber percentage, *PI* protein index, *OI* oil index, *FI* fiber index

Table 2 Predicted genotypic effects expressed as deviations from grand mean for cotton seed traits

Entry	SI	EP	PP	OP	FP	PI	OI	FI
CS-B02	0.279 ± 0.010	−0.659 ± 0.082	0.144 ± 0.026	−0.513 ± 0.063	0.121 ± 0.114	0.071 ± 0.004	−0.006 ± 0.007	0.065 ± 0.015
CS-B04	−0.289 ± 0.020	−0.483 ± 0.044	−0.682 ± 0.058	1.083 ± 0.046	1.318 ± 0.075	−0.144 ± 0.005	0.056 ± 0.005	0.105 ± 0.011
CS-B06	0.171 ± 0.020	0.618 ± 0.041	0.410 ± 0.027	0.154 ± 0.062	0.413 ± 0.130	0.082 ± 0.003	0.049 ± 0.010	0.091 ± 0.018
CS-B07	−0.188 ± 0.016	0.645 ± 0.045	−0.565 ± 0.039	0.038 ± 0.074	0.003 ± 0.062	−0.106 ± 0.006	−0.030 ± 0.007	−0.035 ± 0.010
CS-B16	0.355 ± 0.036	−1.486 ± 0.043	−0.289 ± 0.055	−0.809 ± 0.047	0.211 ± 0.034	0.028 ± 0.012	−0.020 ± 0.010	0.093 ± 0.011
CS-B17	0.746 ± 0.037	1.116 ± 0.059	0.391 ± 0.095	0.751 ± 0.031	−0.165 ± 0.053	0.203 ± 0.020	0.225 ± 0.005	0.111 ± 0.003
CS-B18	−0.240 ± 0.032	0.974 ± 0.107	0.289 ± 0.041	0.909 ± 0.051	−0.636 ± 0.115	−0.006 ± 0.011	0.048 ± 0.010	−0.134 ± 0.012
CS-B25	0.452 ± 0.023	0.614 ± 0.078	−0.072 ± 0.039	0.478 ± 0.030	−0.961 ± 0.053	0.076 ± 0.009	0.137 ± 0.006	−0.043 ± 0.006
CS-B05sh	−0.511 ± 0.026	0.260 ± 0.091	−0.093 ± 0.019	0.182 ± 0.026	−0.942 ± 0.061	−0.109 ± 0.005	−0.078 ± 0.006	−0.207 ± 0.009
CS-B14sh	−0.142 ± 0.028	−0.095 ± 0.048	−0.730 ± 0.044	−0.023 ± 0.044	0.914 ± 0.067	−0.119 ± 0.008	−0.031 ± 0.006	0.077 ± 0.006
CS-B15sh	0.827 ± 0.033	0.539 ± 0.034	−0.786 ± 0.058	0.334 ± 0.045	−0.680 ± 0.044	0.049 ± 0.012	0.200 ± 0.011	0.062 ± 0.003
CS-B22sh	0.562 ± 0.022	0.133 ± 0.032	−0.169 ± 0.104	−1.300 ± 0.089	−0.198 ± 0.061	0.076 ± 0.012	−0.052 ± 0.008	0.080 ± 0.012
CS-B22Lo	−1.108 ± 0.035	−2.173 ± 0.070	−0.034 ± 0.025	−1.820 ± 0.050	0.159 ± 0.051	−0.216 ± 0.009	−0.387 ± 0.010	−0.204 ± 0.006
DP90	−1.546 ± 0.035	−0.904 ± 0.062	−0.563 ± 0.035	0.026 ± 0.023	0.049 ± 0.076	−0.356 ± 0.009	−0.295 ± 0.007	−0.298 ± 0.008
SG747	−1.045 ± 0.034	−1.682 ± 0.068	0.459 ± 0.101	−1.294 ± 0.031	−0.011 ± 0.076	−0.141 ± 0.018	−0.328 ± 0.007	−0.210 ± 0.006
PSC355	−0.771 ± 0.016	1.552 ± 0.034	0.860 ± 0.050	0.482 ± 0.030	−1.152 ± 0.074	−0.047 ± 0.004	−0.099 ± 0.005	−0.284 ± 0.009
ST474	−1.111 ± 0.016	0.388 ± 0.063	0.778 ± 0.046	−0.528 ± 0.018	0.306 ± 0.090	−0.124 ± 0.005	−0.262 ± 0.004	−0.181 ± 0.009
FM966	0.262 ± 0.009	0.620 ± 0.054	0.618 ± 0.067	1.518 ± 0.046	−1.056 ± 0.094	0.134 ± 0.009	0.218 ± 0.005	−0.093 ± 0.009
TM1	0.303 ± 0.016	0.379 ± 0.032	−0.264 ± 0.038	0.674 ± 0.029	−0.139 ± 0.107	0.024 ± 0.006	0.130 ± 0.005	0.030 ± 0.011
3-79	2.994 ± 0.046	−0.355 ± 0.056	0.298 ± 0.030	−0.342 ± 0.054	2.447 ± 0.090	0.624 ± 0.011	0.526 ± 0.013	0.976 ± 0.018
Grand mean	9.845	63.504	20.143	19.865	19.531	1.984	1.959	1.930

SI seed index (g), *EP* embryo percentage (%), *PP* protein percentage (%), *OP* oil percentage (%), *FP* fiber percentage (%), *PI* protein index (g), *OI* oil index (g), *FI* fiber index (g)

3-79 had higher PP, SI, FI, PI, and OI than TM-1 while lower OP and EP than TM-1. The significant difference in genetic effects between a CS-B line and TM-1 is contributed by the substituted chromosome or chromosome arm of 3-79 in TM-1 background.

SI and EP

No CS-B line or cultivar had larger SI than 3-79 (Table 2). Four cultivars DP90, SG747, PSC355, and ST474 had lower SI than TM-1. CS-B17, CS-B25, 15sh, 22sh were higher than TM-1 while CS-B04, 06, 07, 18, 05sh, and 14sh were lower than TM-1 for SI, suggesting these chromosomes or chromosome arms of 3-79 were associated with increased or reduced seed size. DP90 and SG747 had lower EP while PSC355 and FM966 had higher EP than TM-1. CS-B02, CS-B04, CS-B16, CS-B14sh, CS-B22sh, CS-B22Lo had lower EP while CS-B06, CS-B07, CS-B17, CS-B18, CS-B25sh, and CS-B15sh had higher EP than TM-1, indicating that these chromosomes or chromosome arms of 3-79 in TM-1 were closely associated with EP compared to TM-1.

Protein

All commercial cultivars except DP90 had higher PP than TM-1 and 3-79 (Table 2). CS-B02, CS-B06, CS-B17, and CS-B18 had higher PP than TM-1, indicating chromosomes 2, 6, 17, and 18 of 3-79 in TM-1 background were associated with increased protein content compared to TM-1. CS-B04, CS-B07, CS-B14sh, and CS-B15sh had lower PP than TM-1, indicating that chromosomes 4 and 7, and chromosome arms 14sh and 15sh of 3-79 in TM-1 were associated with decreased PP. PI showed a different pattern from PP. Line 3-79 had higher PI than TM-1. DP90, SG747, PSC355, and ST474 had lower PI while FM966 had higher PI than TM-1. CS-B02, CS-B06, CS-B17, and CS-B22sh had higher PI while CS-B04, CS-B07, CS-B05sh, and CS-B14sh had lower PI than TM-1, indicating that chromosomes 2, 6, 17, and chromosome arm 22sh of 3-79 in TM-1 were associated with increased PI while chromosomes 4 and 7, and chromosome arms 5sh and 14sh of 3-79 in TM-1 were associated with decreased PI compared to TM-1.

Oil

All cultivars except FM966 had lower OP than TM-1 (Table 2). DP90, PSC355, and FM966 had higher OP while SG747 and ST474 had higher OP than TM-1. CS-B04 and CS-B18 had higher OP while CS-B02, CS-B06, CS-B07, CS-B16, CS-B05sh, CS-B14sh, CS-B15sh, CS-B22sh, and CS-B22Lo had lower OP than TM-1. The results indicated that chromosomes 4 and 18 from 3-79 in TM-1 were associated with increased OP while chromosomes 2, 6, 7, and 16, and chromosome arms 5sh, 14sh, 22sh, and 22Lo of 3-79 in TM-1 with reduced OP. TM-1, CS-B lines and cultivars had lower OI values than 3-79. CS-B17, CS-B15sh, and FM966 had higher OI while most CS-B lines and the other four cultivars had lower OI than TM-1, indicating most chromosomes or chromosome arms from 3-79 in TM-1 were associated with a reduced IO.

Fiber

No CS-B or cultivar showed higher FP or FI than 3-79 (Table 2). Cultivars PSC355 and FM966 had lower FP than TM-1 while ST474 was higher than TM-1. CS-B04, CS-B06, CS-B16, CS-B14sh, and CS-B22Lo were higher while CS-B18, 25, 05sh, and 15sh were lower than TM-1 regarding FP, suggesting that chromosomes 4, 6, 16, and chromosome arms 14sh and 22Lo of 3-79 in the TM-1 background were associated with increased FP while chromosomes 18 and 25 and chromosome arms 05 and 15sh were associated with reduced FP. All cultivars had lower FI than TM-1. CS-B02, CS-B04, CS-B06, CS-B16, CS-B17, CS-B14sh, CS-B15sh, and CS-B22sh had higher FI while the remaining CS-B had lower FI than TM-1, indicating all these chromosomes or chromosome arms of 3-79 in TM-1 background were associated with FI compared to TM-1.

Correlation between seed traits with agronomic and fiber traits

Correlation among seed traits

SI had high genotypic correlations with PI (0.94), OI (0.91), and FI (0.91) (Table 3). EP was correlated with both OP and OI. The results showed that all index traits tended to be highly correlated with each

other, indicating that large seed size is related to higher PI, OI, and FI. OP and IO were positively correlated (0.55) and FP and FI index were positively correlated (0.73).

Correlations between seed traits with agronomic and fiber traits

LP was negatively correlated with SI (−0.48) and OI (−0.53) but positively correlated with PP (0.53) (Table 4). BW was negatively correlated with FP (−0.48). BN and LY were negatively correlated with SI (−0.61 and −0.62), OI (−0.57 and −0.52), and FI (−0.53 and −0.60) but positively associated with PP (0.49 and 0.50). SI was negatively correlated with MIC (−0.65) while positively with SL (0.59) and T1 (0.60) in these materials. FP, PI, OI, and FI also were

negatively correlated with MIC while positively correlated with fiber length and fiber strength. No significant genotypic correlations among PP, OP, and FP were found. There were significant genetic associations between agronomic and seed traits. In addition, SI, FP, PI, OI, and FI showed negative correlations with micronaire but positive with fiber length and strength. Thus the results suggested that seed traits can be improved simultaneously with fiber quality and yield.

Discussion

As a by-product of lint production, cotton seed which has a good nutrition profile accounts for about 60% of total seed cotton yield. With the increasing demand for food nutrition components and fuel markets, investigation of genetics for cotton seed traits is even more important. Many studies were focused on whole genome based analyzes (Cherry 1983; Kohel et al. 1985; Dani and Kohel 1988; Wu et al. 1995; Wang et al. 1996a, b; Ye et al. 2003). One of the advantages of using chromosome substitution lines is they can be used to associate traits of importance with specific chromosomes (Endrezzi 1963; Kohel et al. 1977; Ma and Kohel 1983; Saha et al. 2004, 2006; Jenkins et al. 2006, 2007; McCarty et al. 2006; Wu et al. 2006). The previous studies on cotton chromosome substitution lines were focused on genetic analyzes of agronomic and fiber traits. However, the investigation of cotton seed traits using cotton chromosome substitution lines has not been reported. By using cotton chromosome substitution lines we were able to

Table 3 Genotypic correlations among seed traits

	EP	PP	OP	FP	PI	OI	FI
SI	0.21	−0.02	0.15	0.40	0.94	0.91	0.91
EP		0.26	0.70	−0.41	0.28	0.46	−0.02
PP			0.01	−0.20	0.30	−0.01	−0.08
OP				−0.26	0.15	0.55	−0.001
FP					0.32	0.23	0.73
PI						0.86	0.85
OI							0.77

A correlation coefficient greater than 0.45 or less than −0.45 indicates significance at probability of 0.05

SI seed index, *EP* embryo percentage, *PP* protein percentage, *OP* oil percentage, *FP* fiber percentage, *PI* protein index, *OI* oil index, *FI* fiber index

Table 4 Genotypic correlations between seed traits and agronomic and fiber traits

	SI	EP	PP	OP	FP	PI	OI	FI
LP	−0.48	−0.25	0.53	−0.29	−0.14	−0.29	−0.53	−0.40
BW	−0.19	0.18	−0.01	0.38	−0.48	−0.20	−0.00	−0.38
BN	−0.61	−0.05	0.49	−0.13	−0.23	−0.42	−0.57	−0.53
LY	−0.62	0.03	0.50	0.02	−0.37	−0.43	−0.52	−0.60
MIC	−0.65	0.04	0.18	0.08	−0.45	−0.58	−0.59	−0.70
SL	0.59	−0.19	0.10	−0.04	0.62	0.61	0.49	0.74
T1	0.60	−0.18	0.16	−0.10	0.56	0.64	0.47	0.72

A correlation coefficient greater than 0.45 or less than −0.45 indicates significance at probability of 0.05

LP lint percentage, *BW* boll weight, *BN* boll number, *LY* lint yield, *MIC* micronaire, *SL* fiber span length, *T1* fiber strength, *SI* seed index, *EP* embryo percentage, *PP* protein percentage, *OP* oil percentage, *FP* fiber percentage, *PI* protein index, *OI* oil index, *FI* fiber index

determine specific chromosome associations with seed traits via comparative analyzes.

Results showed genotypic effects were an important contributor to the phenotypic variances for all seed traits (Table 1). Genotype by environment interactions were significant for SI, EP, OP, and OI. In our previous studies, we found that chromosome arm 5sh was associated with increased flower production (McCarty et al. 2006), chromosomes 16 and 18 with reduced seed cotton yield, chromosome 25 with reduced micronaire, longer fiber, stronger fibers, and chromosome arms 22sh and 22Lo with improved lint percentage (Saha et al. 2004, 2006; Jenkins et al. 2006, 2007; Wu et al. 2006). In this study, chromosome 17 and chromosome arms 15sh and 22sh were strongly associated with increased SI while chromosome 22Lo was strongly associated with reduced seed size, chromosomes 17 and 18 were associated with improved EP while chromosome 16 and chromosome arm 22Lo were associated with reduced EP; chromosomes 6, 17, and 18 were associated with improved PP while chromosomes 4 and 7, and chromosome arms 14sh and 15sh were associated with reduced PP.

Cotton breeders may also be interested in the simultaneous improvement of seed traits, agronomic traits, and fiber traits. Results of this study showed that SI was highly correlated with PI, OI, and FI. No significant genotypic correlations among PP, OP, and FP were found. There were no unfavorable genetic associations between agronomic and seed traits. SI, FP, PI, OI, and FI showed negative correlations with micronaire but positive with fiber length and strength. Thus the results suggested that agronomic traits and seed traits can be improved independently while improving seed traits should also result in the improvement of fiber traits.

Seed yield traits are important seed traits. Seed yield traits were calculated by multiplying seed yield by their seed percentage traits. The data were analyzed by the same model described in this study and the predicted genotypic effects for seed yield, protein yield, oil yield, and seed fiber yield are summarized in Table 5. On average, these 20 lines produced about 1,700 kg ha^{-1} cotton seeds and more than 300 kg ha^{-1} seed protein, oil, and seed fiber accordingly. On average, the five cultivars produced more than 2,000 kg seeds per hectare, and more than

Table 5 Predicted genotypic effects (±SE) (kg ha^{-1}) for seed yield (SY), protein yield (PY), oil yield (OY), and seed fiber yield (FY)

Entry	SY	PY	OY	FY
CS-B02	38.75 ± 7.78	8.59 ± 1.94	3.50 ± 2.03	9.51 ± 2.63
CS-B04	24.87 ± 4.43	−8.17 ± 1.23	18.73 ± 1.04	21.89 ± 1.46
CS-B06	108.19 ± 11.21	26.69 ± 2.24	26.59 ± 2.81	30.20 ± 2.29
CS-B07	79.29 ± 11.67	7.04 ± 2.33	20.50 ± 3.46	13.25 ± 1.72
CS-B16	−514.78 ± 15.86	−112.82 ± 3.24	−119.02 ± 3.11	−90.57 ± 3.57
CS-B17	−57.59 ± 9.83	−6.87 ± 2.81	−5.32 ± 2.05	−13.16 ± 1.57
CS-B18	−245.68 ± 15.01	−51.24 ± 3.00	−45.28 ± 2.57	−45.99 ± 3.55
CS-B25	−36.97 ± 10.55	−9.35 ± 2.22	−3.83 ± 2.10	−17.88 ± 2.25
CS-B05sh	49.80 ± 10.06	10.04 ± 2.36	14.05 ± 1.99	4.57 ± 2.44
CS-B14sh	−181.50 ± 12.56	−47.77 ± 2.73	−38.59 ± 3.25	−22.92 ± 1.88
CS-B15sh	−55.89 ± 7.75	−24.99 ± 1.66	−9.97 ± 1.73	−18.33 ± 1.35
CS-B22sh	−107.82 ± 14.77	−25.03 ± 4.76	−38.03 ± 3.87	−22.66 ± 2.34
CS-B22Lo	−9.99 ± 3.88	−2.82 ± 1.10	−29.13 ± 1.18	−0.21 ± 0.57
DP90	282.02 ± 8.53	47.93 ± 1.35	59.31 ± 1.68	54.83 ± 2.71
SG747	221.27 ± 14.54	58.34 ± 4.87	22.94 ± 2.42	37.48 ± 2.95
PSC355	322.11 ± 12.16	82.68 ± 2.51	80.07 ± 2.76	42.95 ± 2.62
ST474	199.34 ± 9.65	56.64 ± 2.51	34.10 ± 1.79	40.35 ± 2.59
FM966	347.33 ± 14.01	91.34 ± 4.01	100.72 ± 3.18	41.22 ± 1.92
TM1	−26.15 ± 5.97	−10.26 ± 1.17	3.42 ± 1.36	−7.31 ± 2.12
3-79	−436.58 ± 17.90	−89.97 ± 3.28	−94.75 ± 3.92	−57.21 ± 2.86

Table 6 Mean seed yield, protein yield, oil yield, and fiber yield per hectare for CS-B lines, cultivars, TM-1, and 3-79 over four environments

Entry	SY (kg ha^{-1})	PY (kg ha^{-1})	OY (kg ha^{-1})	FY (kg ha^{-1})
CS-B	1,594.73	315.63	319.61	313.99
Cultivars	2,122.13	437.83	428.51	407.89
TM-1	1,661.80	326.97	347.47	321.50
3-79	1,033.31	213.18	205.43	236.45
Average	1,701.86	341.63	342.52	333.97

SY seed yield, *PY* protein yield, *OY* oil yield, *FY* fiber yield

400 kg protein, oil, and seed fiber (Table 6). Even though CS-B lines produced slightly lower seed yield, protein yield, oil yield, and fiber yield than their recurrent parent TM-1, CS-B06, CS-B07, and CS-B05sh produced more seed protein, oil, and seed fiber yield per hectare than TM-1, indicating that chromosomes 6 and 7 and chromosome arm 5sh of 3-79 in TM-1 background are related to improved seed production and seed nutrition production (Table 6). Chromosomes 16, 17, 18, 14sh, 15sh, and 22sh of 3-79 in TM-1, however, are related to reduced seed production and seed nutrition production because CS-B06, CS-B07, and CS-B05sh yielded more seed cotton (Jenkins et al. 2006).

In this study, we investigated cotton chromosome substitution lines regarding their seed nutrition components which have not been previously reported. Significant chromosome associations with these seed traits and their yields were detected in this study. Our study also suggested that seed nutrition traits can be improved with lint yield and fiber quality. Further studies with more complex genetic models should provide additional understanding for these seed traits.

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